G3951

ANTI-TNFα MONOCLONAL ANTIBODY (cA2) PRODUCES ENDOSCOPIC HEALING IN PATIENTS WITH TREATMENT-RESISTANT, ACTIVE CROHN'S DISEASE. GR D'Haens, SJH van Deventer, R Van Hogezand, DM Chalmers, TAJ Brookman, TF Schaible and PJ Rutgeerts. The European cA2 study group in Leuven, Belgium, Amsterdam & Leiden, The Netherlands; Leeds, UK and Centocor, Inc.

Open-label and controlled clinical trials have shown that cA2 reduces the signs and symptoms of Crohn's disease in patients with treatment-resistant, moderate to severe disease activity (van Dulleman *et al*, *Gastroenterology* 1995;109:129, Targan *et al*, *NEJM* 1997;337:1029). To evaluate the relationship of the clinical benefit of cA2 to a reduction in mucosal inflammation, endoscopic response to cA2 was investigated in a multicentre, randomized, double-blind, placebo controlled trial. One hundred eight patients with moderate to severe Crohn's disease (CDAI: 220-400) were studied, 30 of whom were enrolled in Europe and underwent an ileocolonoscopy before and 4 weeks after IV administration of 5 mg/kg (n=7), 10 mg/kg (n=7), 20 mg/kg (n=8) of cA2 or placebo (n=8) as a single 2-hour infusion. The majority of patients were receiving corticosteroids and/or 6-mercaptopurine or azathioprine. Concomitant therapy was kept stable throughout the trial. Video-endoscopic examination was performed at baseline and 4 weeks later after standard bowel preparation by the same endoscopist. Lesions were scored by means of the Crohn's Disease Endoscopic Index of Severity (CDEIS), which was previously validated (Mary and Modigliani, *Gut* 1989;30:983). This score includes the presence of deep/superficial ulceration, ulcerated/non-ulcerated stenosis, and the segments and the proportion of mucosal surface involved by CD.
Significant endoscopic improvement was observed in cA2-treated patients, with a drop in the CDEIS (mean ± SD) from 15.1 ± 6.9 to 6.4 ± 5.1 in the 5 mg/kg group (p=0.006), from 10.6 ± 7.8 to 4.3 ± 5.4 in the 10 mg/kg group (p=0.009), and from 13.3 ± 6.9 to 5.2 ± 2.8 in the 20 mg/kg group (p=0.006). For all cA2 groups combined, the CDEIS dropped from 13.0 ± 7.1 to 5.3 ± 4.4 (p<0.001). There was no endoscopic improvement in the placebo group (CDEIS changed from 8.4 ± 6.3 to 7.5 ± 5.4). The changes in the endoscopic index CDEIS correlated with those in the clinical index CDAI (r=0.56, p=0.002). We conclude that the clinical improvement after cA2-therapy in active Crohn's disease is accompanied by significant healing of endoscopically viewed ileocolonic lesions.
This research was funded by Centocor, Inc., Malvern, PA.

● G3952

EXPRESSION OF INTEGRIN α4β7 ON CIRCULATING AND GUT MUCOSAL LYMPHOCYTES IN INFLAMMATORY BOWEL DISEASE. A Dhiman, L Ang, MJ Weldon, JA Tooze, DJ Ringler* & JD Maxwell. Division of Gastroenterology, St George's Hospital Medical School, London, England UK SW17 0RE. *Leukosite Inc., 215 First Street, Cambridge MA.

Background: In ulcerative colitis (UC) and Crohn's disease (CD), the gut mucosa is infiltrated with increased numbers of activated T and B lymphocytes. The cell adhesion molecule integrin α4β7 is important in the migration of memory T lymphocytes to the gut. Integrin α4β7 is also expressed on B and naïve lymphocytes. Naïve lymphocytes prefer to recirculate through secondary lymphoid tissue such as lymph nodes, but are also recruited to the lamina propria during chronic inflammation. Transfer of CD45RB^{high} naïve T cells into severe combined immunodeficient (*scid*) mice causes colitis.
Aim: To determine if there is a change in the expression of integrin α4β7 on circulating and gut mucosal T (CD3$^+$), B (CD20$^+$), and naïve (CD45RA$^+$) and memory (CD45RO$^+$) lymphocyte subsets in inflammatory bowel disease (IBD).
Method: *Peripheral blood lymphocytes* were separated from venous blood by density gradient centrifugation and *lamina propria lymphocytes* were isolated from 6 colonic biopsies by incubation in collagenase 128u/ml for 3 hours. Lymphocytes were then labelled for dual colour flow cytometry with Act-1 antibody against integrin α4β7 paired with a lymphocyte subset marker. The percentage of each lymphocyte subset expressing integrin α4β7 was determined and the mean values between normal and IBD patients compared for each subset with the unpaired t-test.
Results:

	Percentage of lymphocyte subset expressing integrin α4β7					
	Peripheral blood lymphocytes			*Lamina propria lymphocytes*		
Lymphocyte subset	Control n = 4	Crohn's n = 5	UC n = 11	Control n = 7	Crohn's n = 4	UC n = 11
CD3	68.8	68.4	63.0	38.4	56.2	50.7
CD20	87.1	91.3	89.6	43.0	63.6	60.7*
CD45RA	84.9	85.5	78.7	37.3	50.8	51.8
CD45RO	51.5	44.8	50.7	33.1	43.7	43.8

*p=0.043 for CD20$^+$ subset UC vs Control. No other significant difference between controls and either CD or UC, or between UC and CD in peripheral blood or mucosa.

Conclusion: In inflammatory bowel disease there is no change in the proportion of circulating or mucosal T-cells, memory or naïve lymphocytes expressing integrin α4β7, but more mucosal B lymphocytes express integrin α4β7 in UC. Integrin α4β7 is found on many circulating naïve as well as memory lymphocytes. Integrin α4β7 may therefore play a role in the recruitment of naïve lymphocytes to the gut during chronic inflammation.
Act-1 antibody kindly provided by Leukosite Inc.

G3953

QUANTIFICATION OF *IN SITU* ENDOTHELIAL MUCOSAL ADDRESSIN (MAdCAM-1) EXPRESSION IN INFLAMMATORY BOWEL DISEASE (IBD) USING CONFOCAL MICROSCOPY. A Dhiman, T Poulton*, MJ Weldon, DJ Ringler**, MJ Briskin** & JD Maxwell. Divisions of Gastroenterology and Immunology*, St George's Hospital Medical School, London, England UK SW17 0RE. **Leukosite Inc., 215 First Street, Cambridge, MA.

Background: The endothelial cell adhesion molecule mucosal addressin (MAdCAM-1) is the receptor for the lymphocyte gut-homing integrin α4β7. MAdCAM-1 is present on normal mucosal endothelium and is involved in the extravasation of lymphocytes into mucosal sites. In the murine colitis model of severe combined immunodeficient (*scid*) mice reconstituted with CD45RB^{high} naïve T cells, the expression of MAdCAM-1 is increased on mucosal vessels and blockade of MAdCAM-1 by monoclonal antibodies reduces inflammation. MAdCAM-1 can also be induced on the endothelial cell line bEND.3 by inflammatory cytokines. Confocal laser scanning microscopy allows accurate measurement of fluorescence intensity as the fluorescence emission from a fixed depth of tissue only is analysed.
Aim: To quantify the intensity of endothelial MAdCAM-1 expression in human inflammatory bowel disease.
Method: 5 μm thick sections were made from colonic biopsies taken at colonoscopy from patients with IBD (3 ulcerative colitis, 3 Crohn's disease) and non-inflammatory controls (n=6), and which had been snap frozen in liquid nitrogen. Sections were stained with monoclonal antibody against MAdCAM-1 (clone 10C3) and isotype control antibody using a biotin/streptavidin immunofluorescence technique. The sections were viewed using a confocal microscope and the distribution of fluorescence intensity across 8-10 blood vessels per section was recorded and quantified using Scion Image PC image analysis software. The mean values of endothelial fluorescence intensities were compared between control and IBD subjects using the unpaired t-test.
Results: Mean fluorescence intensity of MAdCAM-1 staining (arbitrary units, +/- SEM) was 48.3 +/- 2.2 for controls and 59.7 +/- 4.0 for IBD. Significant increase in IBD, p=0.031.
Conclusion: MAdCAM-1 is easily detectable on blood vessel endothelium of normal gut mucosa, but MAdCAM-1 expression is increased in IBD. Changes in MAdCAM-1 expression may be important in the increased extravasation of lymphocytes during gut inflammation. Confocal microscopy allows *in situ* measurement of fluorescence intensity and thus may avoid changes in cell if endothelial cells are isolated

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● G3954

CHARACTERIZATION OF MUCOSAL GENE EXPRESSION IN INFLAMMATORY BOWEL DISEASE BY DIRECT HYBRIDIZATION TO MASSIVELY PARALLEL OLIGONUCLEOTIDE ARRAYS. R Dieckgraefe*, W. Stenson*, P. Swanson*, C. Harrington*, and M. Mamades*. *Division of Gastroenterology, Washington University School of Medicine, St. Louis, MO and Affymetrix, Inc., Santa Clara, CA.

Background: Genetic susceptibility plays an important role in the pathogenesis of inflammatory bowel disease (IBD). While many studies have examined the expression of one or a few genes in IBD, no large scale or comprehensive examination of gene expression has been reported. Parallel or high-throughput methods of measuring gene expression have been recently developed which allow concurrent measurement of the expression patterns of a large number of genes. We have utilized the GeneChip® expression monitoring system to examine the mucosal gene expression in ulcerative colitis, Crohn's colitis, and both inflamed and non-inflamed non-IBD specimens. Aims: To identify gene markers differentially expressed in Crohn's disease and ulcerative colitis; identify genotypes associated with particular disease subsets or characteristics (e.g. extent, extraintestinal manifestations, and disease activity) and to begin to establish a catalog of molecules differentially expressed in the context of mucosal inflammation for investigation as potential pharmacological targets. Methods: RNA isolated from the mucosa of colonic resection specimens was used to generate hybridization probes for our analysis. Light-directed solid-phase combinatorial chemistry was used to generate oligonucleotide probe arrays which provide representation of nearly 7000 human cDNA and EST sequences in the form of approximately 260,000 individual 25-mer oligonucleotide elements. Specific hybridization of biotinylated probes was measured by confocal laser scanning after streptavidin phycoerythrin staining. The fluorescence intensity for different levels of gene expression was

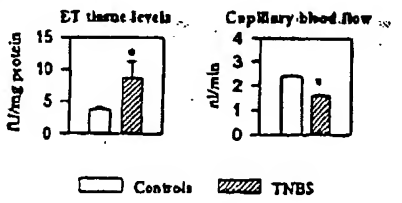
standardized by spiking known amounts of control genes into the probe mixture. Additional tissue samples taken from the area used to isolate RNA were sent for histochemistry. These sections were later scored (in a blinded fashion) by a pathologist for measures of acute and chronic inflammation, dysplasia, eosinophilia, epithelial apoptosis, and metaplastic changes. Results: Hybridization to oligonucleotide arrays was sensitive (detection between 1.5 and 5 pM mRNA), specific and reproducible. Dramatic changes were seen in the expression of a wide range of genes—including cell adhesion molecules, reparative factors, immunoregulatory cytokines, host defense molecules, synthesis of extracellular matrix constituents and matrix degrading molecules, and genes related to B cell maturation and immunoglobulin production. In addition, genes were identified which appear to be specific markers for: a) the specific diagnosis, b) disease activity, and c) specific features of the histology. In addition, there was a suggestion of genotype heterogeneity within the ulcerative colitis group. Conclusions: Oligonucleotide array hybridization provides a sensitive, reproducible method for monitoring differential gene expression in disease tissue. Subclassification by gene expression patterns may improve patient diagnostic classification and identify patients likely to respond to particular forms of therapy. GeneChip arrays and access to the user center were kindly provided by Affymetrix.

G3955

UPREGULATED ACTIVITY OF THE ENDOTHELIN SYSTEM IN EXPERIMENTAL COLITIS.

E. Dickmann[1], T. Foitzik[1], B. Hocher[2], H.J. Buhr[1]. [1]Dept. of Surgery I, Free University Berlin, Germany; [2]Dept. of Medicine V, Humboldt University Berlin, Germany.

Background: Recent studies have suggested that impairment of colonic microcirculation plays an as yet undefined role in the pathogenesis of inflammatory bowel disease (IBD). Effectors of these microcirculatory changes are still unknown. A mediator in this process may be endothelin (ET), a polyfunctional paracrine hormone with microcirculatory effects, which is released by monocytes and macrophages in inflammatory processes. In this study we examined whether impairments of colonic microcirculation are associated with elevated ET-1 tissue levels in early-stage TNBS colitis.
Methods: Colitis was induced in 10 rats by applying 30 mg of TNBS dissolved in 250 µl of 50% ethanol into the distal colon.
After 48 h a minilaparotomy was performed and distal colonic capillary blood flow was determined by intravital microscopy.
Thereafter, distal colon specimens were harvested to measure endothelin tissue levels by ELISA and to perform histological evaluations. Ten healthy rats served as controls.
Results:



Conclusion: Increased ET in colonic tissue in early-stage TNBS colitis may not only be the mediating factor of impaired microcirculation, but could also constitute a direct link to the immunologic and vascular factors in the pathogenesis of IBD. The potential role of ET in colitis is also supported by microcirculatory changes in IBD patients and pathologically elevated ET immunoreactivity of colonic tissue in these cases.

● G3956

HELICOBACTER HEPATICUS DOES NOT POTENTIATE COLITIS IN INTERLEUKIN-10 DEFICIENT MICE.

LA Dieleman[1], SL Tonkonogy[2], RK Sellon[2], RB Sartor[1]. [1]Center for GI Biol. Dis, Univ of N Carolina, Chapel Hill, NC. [2]NSCU College of Vet Med Raleigh, NC.

Mice that lack the interleukin-10 gene develop spontaneous colitis in a specific pathogen-free (SPF) environment, whereas germfree (GF) animals remain disease-free, indicating a role for normal luminal bacteria. In several murine models of experimental intestinal inflammation including IL-10 knockout (KO) mice *Helicobacter hepaticus* has been isolated. This organism can induce colitis and hepatitis in immunodeficient mice, but its role in the development of spontaneous gut inflammation in mice with functioning T lymphocytes remains uncertain. In our study we addressed the effect of *H. hepaticus* during the induction of colitis in IL-10 KO mice. Materials and Methods: GF IL-10 KO mice, 2 months of age, were transferred to a SPF environment. The mice received an oral swab and rectal enema 3 times within 1 week with either stool from *H. hepaticus* positive or *H. hepaticus* negative animals. Mice were sacrificed on either day 7 or day 17 post SPF-induction. PCR was performed on DNA isolated from cecal contents using specific primers to assess the presence or absence of *H hepaticus*. Histology from various parts of large intestine was blindly scored for the amount of inflammation using a validated scale. Mesenteric lymph node cells were assessed for cell numbers, proliferation with media alone, LPS, Con A and anti-CD3 using 3H thymidine incorporation as well as quantitation of the activation markers L-selectin, CD44 and CD45RB using FACS analysis. IL-12 concentrations were measured in colon cultures using a specific ELISA.
Results:
Histology scores:

Groups	*cecum*	*dist colon*	*cecum*	*dist colon*
	day 7	*day 7*	*day 17*	*day 17*
H. hepaticus-neg.	2.4* 0.4	1.3* 0.7	2.9* 0.6	2.2* 0.8
H. hepaticus-pos.	2.6* 0.2	1.4* 0.8	2.9* 0.5	1.8* 0.2

Presence of *H hepaticus* was shown by PCR. Mice in both groups developed colitis in cecum and distal colon within 7 days of SPF conditions. There were no significant differences in weight loss, nor in histological scores at either 7 days or 17 days post-SPF colonization in the absence or presence of *H. hepaticus*. Cell numbers, proliferation indices and activation markers of MLN cells from both groups showed no significant differences nor did the IL-12 concentrations in colon cultures differ between the groups. Conclusions: IL-10 KO mice transferred from GF to SPF conditions develop colitis, even in the absence of *H. hepaticus*. The presence of *H. hepaticus* has no effect in the development of SPF-induced colitis in this model. *H. hepaticus* does not appear to influence chronic intestinal inflammation in mice with functioning T lymphocytes.

● G3957

EPITHELIAL NITRIC OXIDE EXPRESSION IN INFLAMMATORY BOWEL DISEASE: AN OXIDATIVE BARRIER OF THE INFLAMMED MUCOSA?

G. Dijkstra, H.M. van Dullemen, H. Moshage, A. de Jager-Krikken, A.T.M.G. Tiebosch, P.L.M. Jansen, H. van Goor. Depts. of Gastroenterology and Pathology, University Hospital, Groningen, The Netherlands.

Background: Small amounts of nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) is thought to be protective in maintaining microvascular integrity and in inhibiting both platelet aggregation and leukocyte adhesion. High concentrations of NO, as produced by inducible nitric oxide synthase (iNOS), can be direct or indirect cytotoxic in its reaction with superoxide anions ($O_2^{\cdot-}$) yielding peroxynitrite ($ONOO^-$). Toxic effects of $ONOO^-$ on tissue can be visualized as nitrotyrosine. In addition NO and $ONOO^-$ has antibacterial properties and may have a protective role in inhibiting bacterial translocation. *Aim:* To study the activation of iNOS and the presence of NO mediated tissue damage. *Methods:* Colonic mucosal biopsies from 7 controls, 10 patients with active ulcerative colitis (UC) and 10 patients with active Crohn's disease (CD) were stained with commercial antibodies against eNOS, iNOS and nitrotyrosine. $O_2^{\cdot-}$ producing cells were detected cytochemically. *Results:* iNOS was strongly expressed in epithelial cells of inflamed mucosa of all UC and CD patients but not in non-inflamed mucosa of IBD patients and controls. Cells staining for $O_2^{\cdot-}$ were sparsely present in the lamina propria of controls. Actively inflamed mucosa showed a high expression of $O_2^{\cdot-}$ positive cells in the lamina propria. All $O_2^{\cdot-}$ positive cells were also nitrotyrosine positive. However, there were no nitrotyrosine residues in or near iNOS positive epithelial cells. The eNOS expression in intestinal biopsies of IBD patients was unaltered.
Conclusions: The high epithelial iNOS expression in actively inflamed mucosa of IBD patients appears not to be associated with nitrotyrosine formation. Nitrotyrosine formation is confined to an area with a high expression of $O_2^{\cdot-}$ producing cells. Therefore NO from epithelial iNOS may function as an oxidative barrier at the sites where the mucosa is severely inflamed.